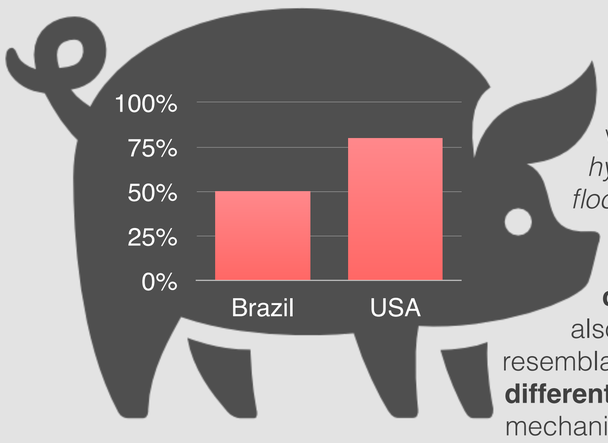
Metabolic Investigation of the Mycoplasmas from the Swine Respiratory Tract

M.G. Ferrarini[1,2,3], F.M. Siqueira[2], S.G. Mucha[2], T.L. Palama[4,5], E. Jobard[6], B.E. Herrmann[4,6], A.T.R Vasconcelos[7], F. Tardy[8,9], I.S. Schrank[2], A. Zaha[2], M.F. Sagot[1,3]

DOI: 10.1186/s12864-016-2644-z

1. ERABLE, Inria, 43, Bd du 11 Novembre 1918, Villeurbanne, France.
2. CBiot, UFRGS, Av Bento Gon'calves, Porto Alegre, 9500, Brazil.
3. Laboratoire de Biométrie et Biologie Évolutive, Université de Lyon, 43, Bd du 11 Novembre 1918, Villeurbanne, France.
4. Université de Lyon, Institut des Sciences Analytiques (CNRS, ENS Lyon, Université Lyon 1), 5, Rue de la Doua, Villeurbanne, France.
5. Current address: LISBP - INSA Toulouse, Toulouse, France.
6. Université de Lyon, Centre Léon Bérard, Département d'oncologie médicale, 28, rue Laënnec, Lyon, France.
7. Laboratório Nacional de Computaćão Científica, Av. Getúlio Vargas, 333, Petrópolis, Brazil.
8. Anses, Laboratoire de Lyon, UMR Mycoplasmoses des Ruminants, 31, Av Tony Garnier, Lyon, France.
9. Université de Lyon, VetAgro Sup, UMR Mycoplasmoses des Ruminants, 1 Avenue Bourgelat, Marcy L'Étoile, France.

Introduction



The respiratory tract of swine is colonized by several bacteria among which are three *Mycoplasma* species: *Mycoplasma flocculare*, *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis*. While colonization by *M. flocculare* is virtually asymptomatic, *M. hyopneumoniae* is the causative agent of enzootic pneumonia and *M. hyorhinis* is present in cases of pneumonia, polyserositis and arthritis [1]. *M. hyopneumoniae* is considered a **major cause of economic loss in the pig industry (Fig. 1)** [2] and *M. hyorhinis* is also a well-known contaminant of mammalian cell cultures [3]. The genomic resemblance among these three *Mycoplasma* species [4] combined with their **different levels of pathogenicity (Fig. 2)** is an indication that they have unknown mechanisms of virulence and differential expression, as for most mycoplasmas. In this work, we reconstructed whole-genome metabolic networks for these three mycoplasmas and performed metabolomic experiments through nuclear magnetic resonance spectroscopy (NMR) with **selected strains (Fig. 3)** to acquire experimental data and further refine the models.

Figure 1. Estimated prevalence of *M. hyopneumoniae* in Brazilian and American herds.
Sources: Vicente, *et al.*, 2013; Ross, 1992.

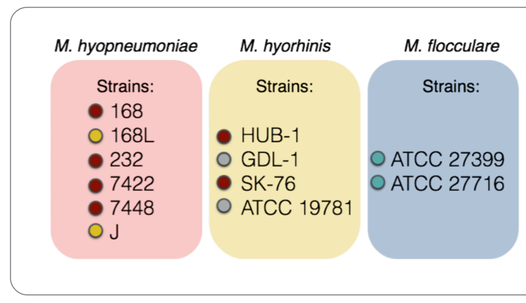


Figure 2. Different levels of pathogenicity between species and within selected strains of *M. hyopneumoniae*, *M. hyorhinis* and *M. flocculare*.

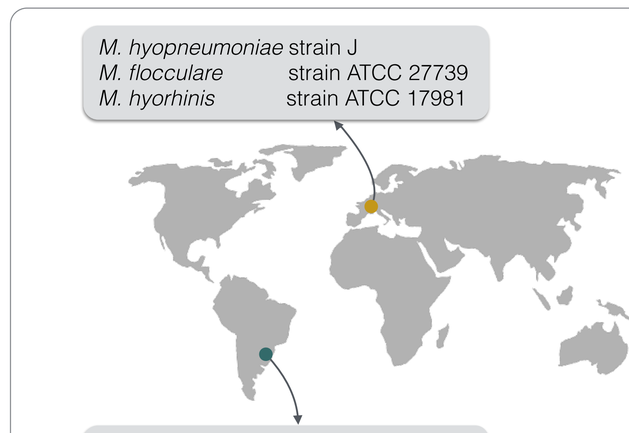


Figure 3. Experimental availability of selected strains in Brazil and France.

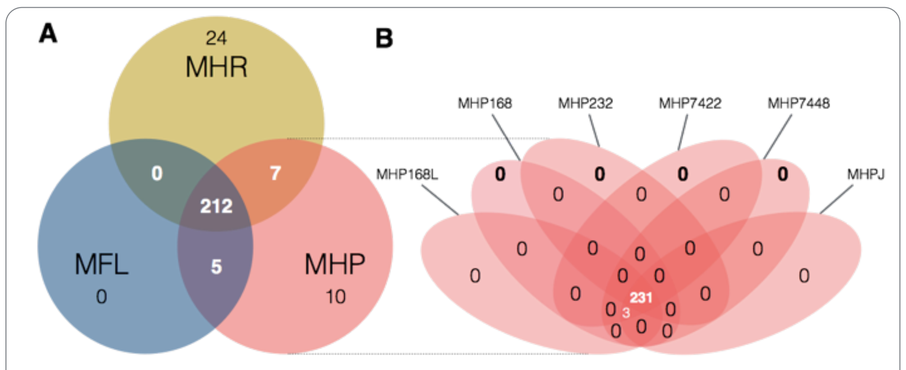


Figure 4. Venn diagrams representing the comparison of refined networks. Numbers represent the exclusive and common reactions present in the refined networks **(a)** between species, and **(b)** between selected strains of *M. hyopneumoniae*. This analysis shows that most of the metabolism is common to all organisms. MHR: *M. hyorhinis*; MHP: *M. hyopneumoniae*; MFL: *M. flocculare*.

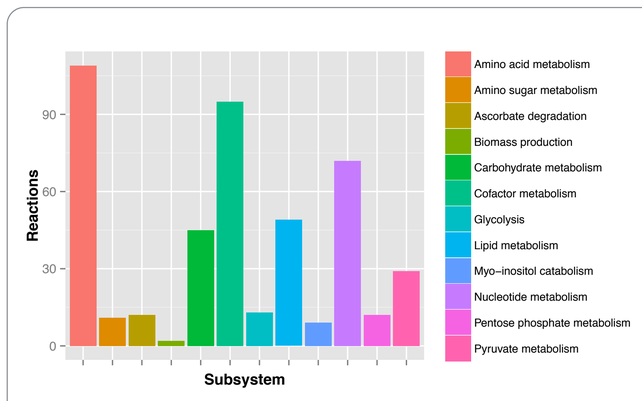


Figure 5. Distribution of the model reactions in the subsystems. The 457 reactions present in the model iMFpan were separated into metabolic subsystems.

Results

Even though the refined models have **similar metabolic capabilities (Fig. 4)**, **interesting differences include (Table 1)** a wider range of carbohydrate uptake in *M. hyorhinis*, which in turn may also explain why this species is a contaminant widely present in cell cultures. In addition, the myo-inositol catabolism is exclusive to *M. hyopneumoniae* and may be an important trait for virulence. However, the most important difference seems to be related to glycerol conversion to dihydroxyacetone-phosphate (comprised within lipid metabolism), which produces toxic hydrogen peroxide. This activity, missing only in *M. flocculare*, may be directly involved in cytotoxicity, as already described for two lung pathogenic mycoplasmas, namely *Mycoplasma pneumoniae* in human and *Mycoplasma mycoides* subsp. *mycoides* in ruminants [5,6]. **Metabolomic data (Fig. 6)** suggest that even though these mycoplasmas are extremely similar in terms of genome and metabolism, distinct products and reaction rates may be the result of differential expression throughout the species.

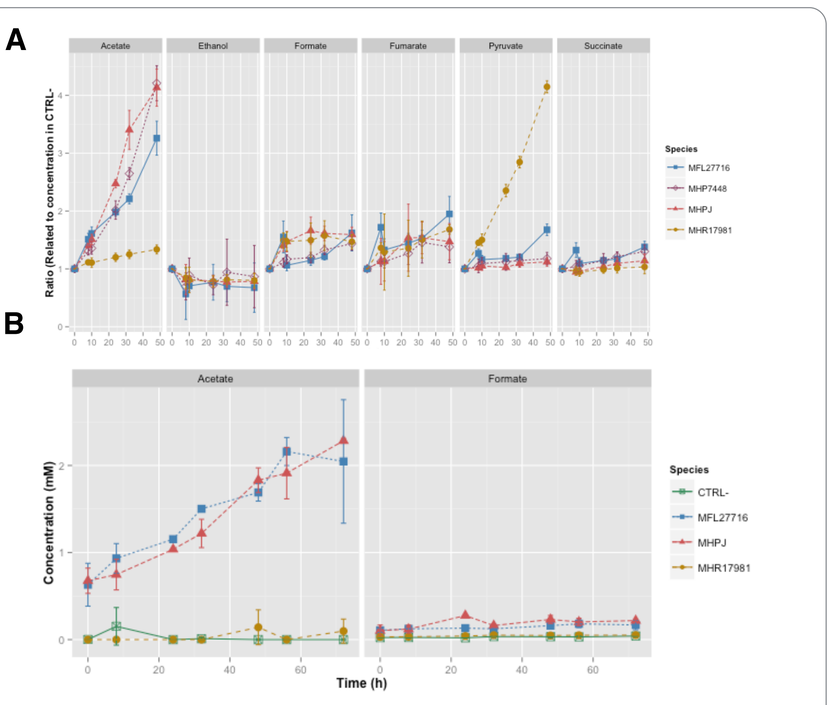


Figure 6. Distinct products of the metabolism of pyruvate from growth of *M. hyopneumoniae* (MHPJ, MHP7448), *M. flocculare* (MFL27716) and *M. hyorhinis* (MHR17981). In complex medium, we calculated the ratio between the peak signal in cultivated versus control medium and error bars were calculated as the standard deviation between triplicate time-matched samples. For defined medium, we detected the actual concentration for the metabolites and error bars were calculated as the standard deviation between duplicate time-matched samples. **(a)** In complex medium, *M. hyopneumoniae* (both strains) and *M. flocculare* can produce high amounts of acetate; the yields are even higher from *M. hyopneumoniae*. *M. hyorhinis*, on the other hand, produces low concentrations of acetate in this medium. The final glycolysis product for *M. hyorhinis* is thus pyruvate. **(b)** In defined medium, *M. hyopneumoniae* (strain J) and *M. flocculare* produce similar amounts of acetate while *M. hyorhinis* contains only residual levels of this metabolite. The three species can produce low amounts of formate in both media

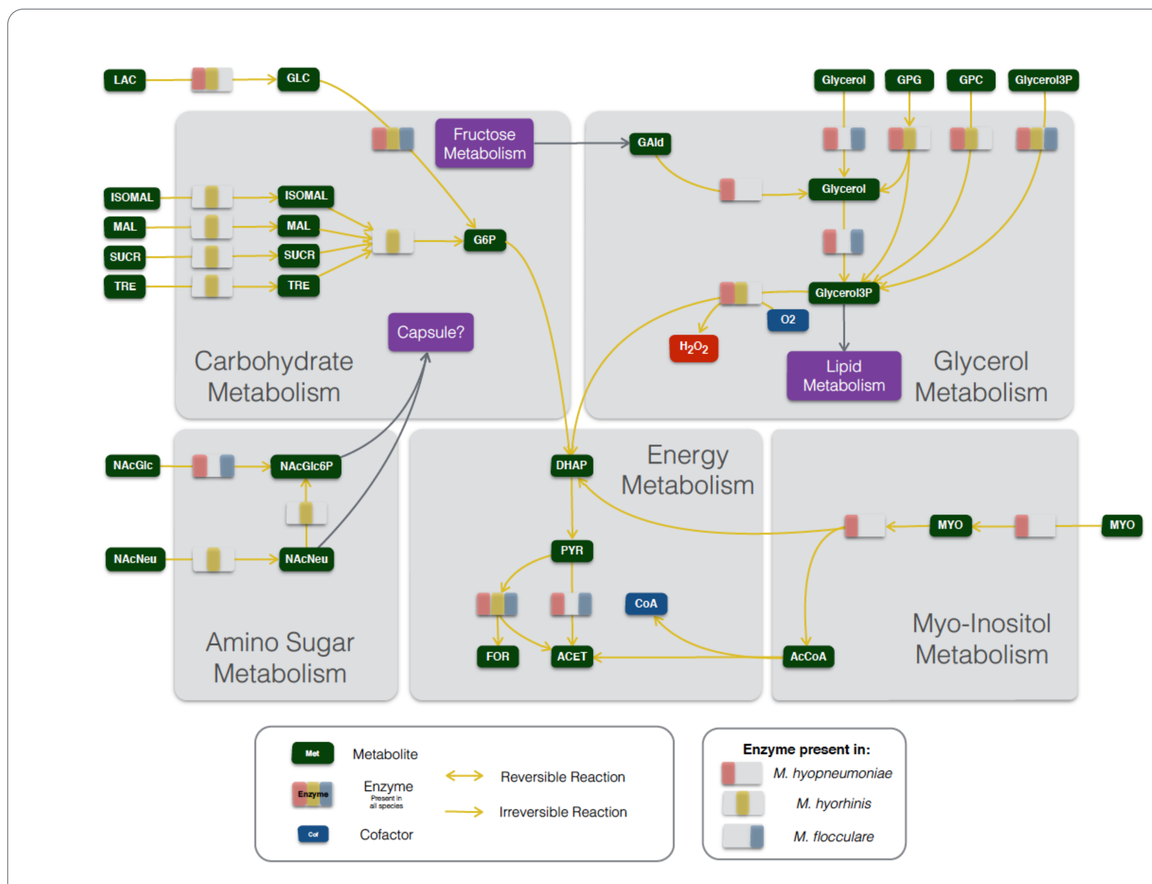


Figure 7. Differential metabolism of *M. hyorhinis*, *M. hyopneumoniae* and *M. flocculare*. Metabolites are depicted in dark green and separate enzymatic activities for *M. hyorhinis*, *M. hyopneumoniae* and *M. flocculare* can be seen in yellow, pink and blue, respectively. Whenever an enzyme is missing from the three species, the enzyme rectangle is depicted in grey.

Table 1. Gene-Protein-Reaction (GPR) exclusive associations for each species networks distributed in subsystems. Model names species-specific are as follows: iMFmhr for *M. hyorhinis*; iMFmhp for *M. hyopneumoniae* and iMFmfl for *M. flocculare*.

Number of Exclusive GPRs			
Subsystem	**iMFmhp**	**iMFmhr**	**iMFmfl**
Aminoacid metabolism			
Amino sugar metabolism		5	
Glycolysis			
Pentose Phosphate Pathway			
Ascorbate degradation			
Carbohydrate Metabolism		13	
Myo-inositol degradation	7		
Pyruvate Metabolism	2		
Lipid metabolism	1	2	
Nucleotide Metabolism		1	
Cofactor metabolism		3	
Total	**10**	**24**	**0**

Conclusions

We were able to infer from the reconstructed networks that the lack of pathogenicity of *M. flocculare* if compared to the highly pathogenic *M. hyopneumoniae* may be related to its incapacity to produce cytotoxic hydrogen peroxide. Moreover, the ability of *M. hyorhinis* to grow in diverse sites and even in different hosts may be a reflection of its enhanced and wider carbohydrate uptake. Altogether, the **metabolic differences highlighted *in silico* and *in vitro* (Fig. 7)** provide important insights to the different levels of pathogenicity observed in each of the studied species.

References

1. Kobisch M, Friis NF. Swine mycoplasmoses. Rev - Off Int Epizoot. 1996; 15(4):1569–605.
2. Maes D, Verdonck M, Deluyker H, de Kruif A. Enzootic pneumonia in pigs. Vet Q. 1996; 18(3): 104–9.
3. Nikfarjam L, Farzaneh P. Prevention and detection of Mycoplasma contamination in cell culture. Cell J. 2012; 13(4):203–12.
4. Siqueira FM, Thompson CE, Virginio VG, Gonchoroski T, Reolon L, Almeida LG, da Fonseca MM, de Souza R, Prosdocimi F, Schrank IS, Ferreira HB, de Vasconcelos AT, Zaha A. New insights on the biology of swine respiratory tract mycoplasmas from a comparative genome analysis. BMC Genomics. 2013; 14:175.
5. Vilei EM, Frey J. Genetic and biochemical characterization of glycerol uptake in mycoplasma mycoides subsp. mycoides SC: its impact on H(2)O(2) production and virulence. Clin Diagn Lab Immunol. 2001; 8(1):85–92.
6. Hames C, Halbedel S, Hoppert M, Frey J, Stulke J. Glycerol metabolism is important for cytotoxicity of Mycoplasma pneumoniae. J Bacteriol. 2009; 191(3):747–53.

Finantial Support:
ERC SYSYPHE